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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Graham P. Allaway, et al,	
U.S. Serial No.:	09/412,284	Examiner: J. Parkin
Filed:	October 5, 1999	Group Art Unit: 1648
For:	Methods For Using Resonance Energy Transfer-Based Assay of HIV-1 Envelope Glycoprotein-Mediated Membrane Fusion, and Kits For Practicing Same	

Assistant Commissioner for Patents
Washington. D.C. 20231

SIR:

Declaration Under 37 C.F.R.§1.132 of Paul J. Maddon, M.D.,Ph.D.

I, Paul J. Maddon, M.D., Ph.D., hereby declare that:

1. I am a founder and Chief Executive Officer of Progenics Pharmaceuticals, Inc. in Tarrytown New York. I received an M.D./Ph.D. in 1988 from Columbia University and have 20 years experience working in the monoclonal antibody field. A copy of my curriculum vitae is attached hereto as **Exhibit 1**.

2. I am a coinventor of the subject matter disclosed and claimed in the above-identified patent application.

3. My expertise and experience in making, characterizing and using monoclonal antibodies includes over 31 peer reviewed scientific publications since 1985. My research includes generating, using and characterizing monoclonal antibodies against various antigens and epitopes associated with cell surface antigens, cell surface receptors, and HIV-1 envelope glycoprotein. My research also includes generating and using monoclonal antibodies in cancer detection and treatment. Thus, I am very familiar with the field of making monoclonal antibodies and knowledgeable about the level of skill of those active in the field.

4. I understand the claimed invention to be an antibody capable of specifically inhibiting the fusion of a macrophage tropic primary isolate of HIV-1 with a CD4+ cell susceptible to infection by a macrophage-tropic primary isolate of HIV-1 as well as an antibody determined to be capable of specifically inhibiting the fusion of a macrophage-tropic primary isolate of HIV-1 to a CD4+ cell, but not a T cell-tropic isolate of HIV-1 to a CD4+ cell, using a method which comprises:

(a) contacting (i) a first appropriate CD4+ cell, which is labeled with a first dye, with (ii) a cell expressing the HIV-1 envelope glycoprotein of the macrophage-tropic primary isolate of HIV-1 on its surface, which is labeled with a second dye, in the presence of an excess of the antibody under conditions which would normally permit the fusion of the CD4+ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of the antibody, the first and second dyes being selected so as to allow resonance energy transfer between the dyes;

(b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and

(c) determining whether there is a reduction of resonance energy transfer, when compared with the resonance energy transfer in the absence of the antibody;

(d) contacting (i) a second appropriate CD4+ cell, which is labeled with a first dye, with (ii) a cell expressing the HIV-1 envelope glycoprotein of a T cell-tropic isolate of HIV-1 on its surface, which is labeled with a second dye, in the presence of an excess of the antibody under conditions which would normally permit the fusion of the CD4+ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of the antibody, the first and second dyes being selected so as to allow resonance energy transfer between the dyes;

(e) exposing the product of step (d) to conditions which would result in resonance energy transfer if fusion has occurred;

(f) determining whether there is a reduction of resonance energy transfer, when compared with the resonance energy transfer in the absence of the antibody; and

(g) comparing the determination made in step (c) with the determination made in step (f), wherein a decrease in transfer in step (c) but not in step (f) indicates that the antibody is capable of specifically inhibiting fusion of the macrophage-tropic primary isolate of HIV-1 to CD4+ cells, but not capable of specifically inhibiting the fusion of a T cell-tropic isolate of HIV-1 to the CD4+ cells.

Accordingly, the claimed invention relates to an antibody having well defined, specific characteristics, and uses thereof.

5. I understand that the claimed invention has been rejected by the United States Patent and Trademark Office on the basis that the specification allegedly does not enable one skilled in the art to make and use the invention.

6. It was standard practice as of June 7, 1996 for one skilled in the art to make an antibody by immunizing an animal with a particular immunogen . The technology of antibody production was widely used and highly predictable as of June 7, 1996.

7. Based on the disclosure in the present patent application and the general knowledge in the field of making antibodies as of June 7, 1996, one skilled in the art could have readily made and used an antibody such as is defined in paragraph 4 above. Making antibodies to cell surface antigens including cell surface receptors on whole cells was a well defined technology as of June 7, 1996. See Kohler and Milstein (1975) (**Exhibit 2**). As of June 7, 1996, the level of skill of one of ordinary skill in the art of making an antibody was a laboratory technician with a bachelor's degree and one to two years of experience working with hybridomas. Such a person of ordinary skill could have readily made an antibody such as is recited in the claims prior to June 7, 1996.

8. The present specification teaches a straightforward, reproducible method for making and identifying an antibody of the present invention. The application describes, among other things, the following: a source of an immunogen for eliciting an antibody of the present invention (PM1 cells, page 60, lines 9-11); a method for obtaining an antibody by recovering supernatant from hybridomas generated by immunizing with PM1

cells as the immunogen (page 60, lines 9-13); and a differential screening assay called the resonance energy transfer ("RET") assay for identifying an antibody having the desired characteristics (pages 30 through 32, line 15). These characteristics include the ability of the antibody to inhibit HIV-1 envelope glycoprotein-mediated membrane fusion using HeLa cells expressing envelope glycoprotein derived from a macrophage-tropic HIV-1 isolate, HIV-1 $_{JR-FL}$ ("HeLa-env $_{JR-FL}$ cells") while not inhibiting HeLa cells expressing envelope glycoprotein derived from a T cell-tropic HIV-1 isolate, HIV-1 $_{LAI}$ ("HeLa-env $_{LAI}$ cells"). Antibodies generated by immunizing with CD4+ PM1 cells inhibited fusion between HeLa-env $_{JR-FL}$ cells and CD4+ PM1 cells (page 60, lines 11-16). This demonstrates that using PM1 or equivalent cells as the immunogen and the RET assay as the differential screening assay, one skilled in the art is able to readily make, identify and select antibodies that have the characteristics of inhibiting fusion between CD4+ PM1 cells and HeLa-env $_{JR-FL}$ cells. Table 3 on page 61 demonstrates that fusion-inhibiting antibodies react with an antigen on the surface of a PM1 cell, do not react with CD4, and do not crossreact with an antigen on the surface of a SUP-T1 cell. Accordingly, the application provides detailed guidance and direction for making an antibody and selecting an antibody having the desired characteristics of inhibiting the fusion of a macrophage-tropic primary isolate of HIV-1 to a CD4+ cell but not a T cell-tropic isolate of HIV-1 to a CD4+ cell.

9. It is not necessary for one of ordinary skill in the art to know the antigenic determinants or epitopes on the surface of the whole cells used for immunization or their structural configuration in order to make an antibody having the characteristics of the

antibody claimed in the present invention. It is well established that having a starting immunogenic source such as an immunogenic whole cell and following standard immunization methods, a series of antibodies will be elicited. It is also well established that the screening method allows one skilled in the art to identify and select antibodies having the desired characteristics. The RET assay measures the rate of fusion of a CD4+ cell membrane with HIV-1 envelope glycoprotein+ cell membrane. By using different sources of HIV-1 envelope glycoprotein, i.e., from a macrophage-tropic primary isolate of HIV-1 or from a T cell-tropic isolate of HIV-1, in combination with different sources of CD4+ cells, i.e., primary human macrophage or from a human T lymphocyte, the RET assay as disclosed in the specification is an exquisitely sensitive assay for identifying an antibody capable of specifically inhibiting fusion of a macrophage-tropic primary isolate of HIV-1 with a CD4+ cell. One of ordinary skill in the art using the screening methods disclosed in the subject application would readily obtain an antibody having the characteristics claimed in the present invention.

10. Using methods as disclosed in the present specification, my colleagues and I made additional antibodies that inhibited HIV-1 envelope –mediated fusion between HeLa-$env_{JR\text{-}FL}$ cells and the CD4+ cells, PM1 cells, in a RET assay. The results were reported in the *Journal of Virology*, May 1999, p. 4145-4155 (**Exhibit 3**). Of 10,000 hybridoma supernatants screened in the RET assay, over 100 inhibited fusion by greater than 50% (page 4146, first column, paragraph 3; page 4146, second column, paragraph one). Six antibodies positive in the RET assay, designated therein as PA8, PA9, PA10, PA11, PA12 and PA14,

were further characterized. All of the antibodies bind to an antigen which is found on the surface of the macrophage cell line, PM1.

11. Thus, the present specification provides sufficient teaching to one skilled in the art to readily enable making, screening, and selecting antibodies that specifically inhibit fusion of a macrophage-tropic primary isolate of HIV-1 to a CD4+ cell susceptible to infection by a macrophage-tropic primary isolate of HIV-1 without undue experimentation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.

DATE: March 7, 2002 Paul J. Maddon

Paul J. Maddon, M.D., Ph.D.